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Evidence for a Mechanism of Demyelination by Human JC Virus: Negative Transcriptional Regulation of RNA and Protein Levels From Myelin Basic Protein Gene by Large Tumor Antigen in Human Glioblastoma Cells

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Human JC virus (JCV) is a neurotropic human polyomavirus that was found in the plaques and oligodendroglial cells of the brains of patients with the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). Transgenic mice expressing JCV large tumor (T)-antigen from integrated DNA showed dysmyelination in the central nervous system. However, the role of T-antigen from episomal DNA in the demyelination in PML remains unclear. In this report, we examined the effect of episomally expressed JCV T-antigen on the expression of myelin basic protein (MBP) in U-87 MG human glioblastoma cells to study the mechanism of demyelination. Expression assays of the MBP promoter in U-87 MG detected a 2.5-fold reduction in cells expressing intact T-antigen. Next, U-87 MG expressing T-antigen were examined by RNase protection assays for mRNA accumulation from the endogenous MBP promoter. Also, the expression of the MBP promoter plasmid was determined using in vitro transcription assays with extracts from T-antigen expressing cells. Both assays found a similar down-regulation of the MBP promoter by T-antigen, confirming that negative regulation occurred at the transcriptional level for the endogenous and exogenous MBP promoters. Furthermore, in situ immunofluorescence assays and quantitative Western blot analysis provided convincing evidence of a similar reduction in the level of MBP produced from the functional endogenous gene in U-87 MG glioblastoma cells expressing T-antigen. Thus, we provide evidence for the role of T-antigen in a transcriptional control mechanism for the demyelination that is caused by JCV in PML patients.

KEY WORDS: neurotropism, brain, transcriptional regulation, protein accumulation, progressive multifocal leukoencephalopathy, mRNA accumulation

INTRODUCTION

The function of the central nervous system (CNS) requires the ability to rapidly conduct impulses, which is dependent on the integrity of the myelin sheath that is wrapped around the axon membranes by oligodendrocytes. Myelin basic protein (MBP) is an important member of the major group of myelin proteins that stabilize the myelin membrane [Demel et al., 1973]. MBP is produced in the peripheral nervous system (PNS) by Schwann cells and in the CNS by oligodendrocytes [Brophy et al., 1993]. The importance of MBP is clearly demonstrated by the recessive murine mutation called shiverer. Myelin in the brains of these mice is almost undetectable, apparently due to the deletion of most of the MBP gene [Roach et al., 1983]. The effects of this mutation include various neurological disorders that culminate in death. Similar demyelinated lesions were observed within the white matter in the autopsies of the CNS for the patients who succumb to the fatal brain disease called progressive multifocal leukoencephalopathy (PML) [Åström et al., 1958].

Human JC polyomavirus (JCV) is strictly neurotropic and replicates only in glial cells. The plaques of PML brains were shown to contain JCV and the oligodendrocytes that surrounded the plaques were shown to be infected by JCV [Padgett et al., 1977; Johnson, 1983]. Therefore, the demyelination that is observed in the

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CNS lesions of PML patients is thought to result from an expanding lytic JCV infection of the surrounding myelin-producing oligodendrocytes. PML became more prevalent due to the immunosuppressive chemotherapy that is used during organ transplantation. More recently, the incidence of PML has grown proportionately to the incidence of acquired immunodeficiency syndrome (AIDS), with which PML is associated [Quinlivan et al., 1992; for review see Major et al., 1992].

Previously, transgenic mice expressing JCV large tumor (T)-antigen were used to suggest the importance of this protein for the induction of PML by JCV [Small et al., 1986; Haas et al., 1994]. However, these transgenic mice contained JCV T-antigen DNA that was linearly integrated and continually expressed in the mouse cells. Therefore, the relevance to the MBP in the human oligodendroglial cells that are infected with and expressing circular episomal JCV DNA only during the lytic cycle is uncertain. We transiently transfected circular episomal JCV T-antigen DNA into U-87 MG human glioblastoma cells to assay the effect on expression from the MBP promoter. The effect of JCV T-antigen expression was the proportionally similar down-regulation of the expression of the MBP gene in four functions: functional transcription, level of mRNA accumulation, rate of mRNA synthesis, and level of protein. Thus, the down-regulation by T-antigen of the MBP gene at the transcriptional level may explain the demyelination that is observed for PML.

MATERIALS AND METHODS

Plasmids, Cell Culture, and Transient Expression Assays

The following T-antigen expression plasmids were described previously: CMV-JCV-TΔ, which contains a deletion of JCV T-antigen from nucleotide (nt) position 4958 to 3013; CMV-JCV-TA, which contains intact T-antigen; and JCV-pML, which contains the intact JCV genome plasmid [Nakshatri et al., 1990]. The pMBPcat MBP promoter-enhancer expression plasmid was prepared by inserting the 1.3 kb *Hind*III fragment from the −1.3 kb MBP plasmid [Miura et al., 1989] into the *Hind*III site of the promoter-enhancerless pSV0cat vector [Gorman et al., 1982] (Fig. 1A). U-87 MG human glioblastoma cells (ATCC 14-HTB) were transfected with DNA, chloramphenicol acetyltransferase (CAT) transient expression was assayed, and the CAT values were normalized with assays of the cotransfected internal control, pRSV-β-gal RSV promoter-β-galactosidase plasmid, as described previously [Kumar et al., 1993].

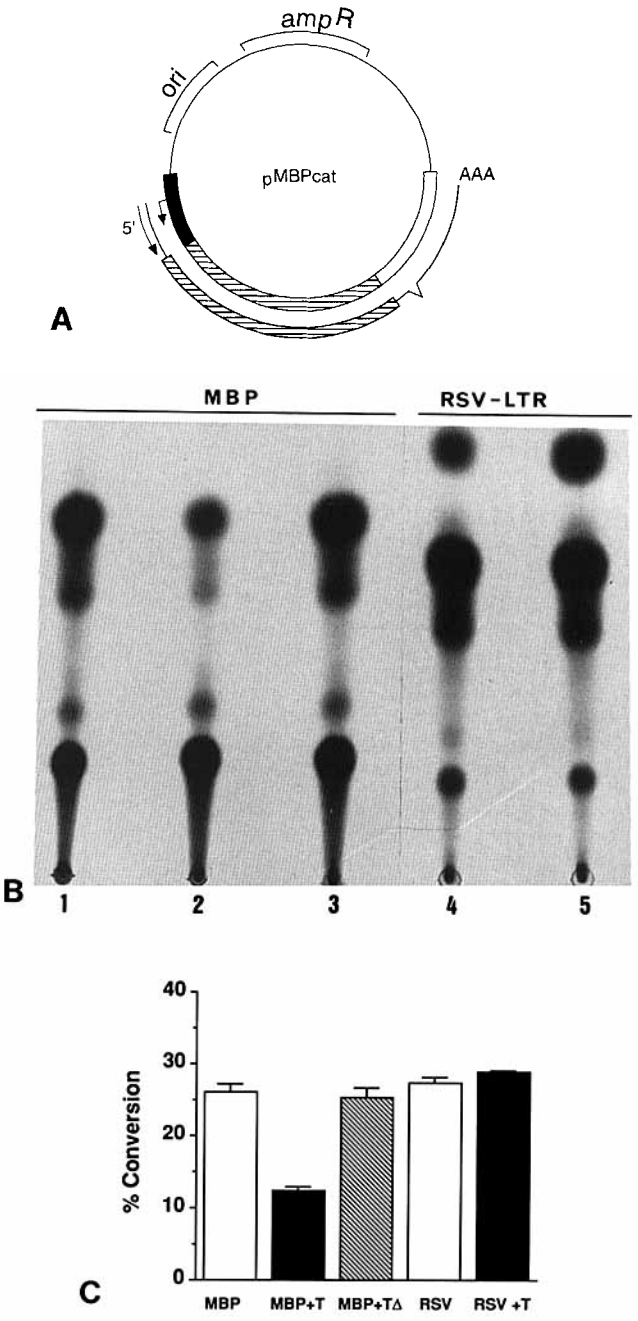

Fig. 1. Effect of JCV T-antigen on functional MBP promoter expression from a reporter plasmid in human glioblastoma cells. **A:** Diagram of the pMBPcat reporter plasmid. the black box represents the inserted MBP promoter; the boxes with diagonal lines represent the chloramphenicol acetyltransferase (CAT) coding sequences; the outer semicircle represents the mRNA; the lines in the outer semicircle represent the 5′ and 3′ untranslated regions; AAA indicates the poly(A) sequences; the arrows indicate the initiation site and direction of transcription; and ori and amp® indicate plasmid sequences. The diagram is not drawn to scale. **B:** Negative regulation of the function of the MBP promoter by JCV T-antigen in U-87 MG. The cells were transfected with 2 μg pMBPcat MBP (MBP) promoter reporter plasmid only **(lane 1)** or were contransfected with 10 μg CMV-JCV-TA T-antigen expressing plasmid **(lane 2)** or CMV-JCV-TΔ deleted T-antigen expressing plasmid **(lane 3).** As a control for the efficiencies of transfection and expression, the cells were transfected with 2 μg pRSV-β-gal (RSV-LTR) expression plasmid only **(lane 4)** or were contransfected with 10 μg CMV-JCV-TA **(lane 5).** The total level of DNA was adjusted to 20 μg with pUC19. The upper three spots are the CAT-acetylated products. **C:** Graph of the quantified data for B. The CAT activity was determined from assays with less than 30% conversion and were normalized with assays for the pRSV-β-gal RSV promoter-β-galactosidase plasmid contransfected control. Results are the averages of three experiments and were quantified by liquid scintillation counting. The mean and standard deviation are shown for pMBPcat alone (MBP), with CMV-JCV-TA (MBP + T), with CMV-JCV-TΔ (MBP + TΔ), with pRSV-β-gal (RSV), and with RSV plus CMV-JCV-TA (RSV + T).

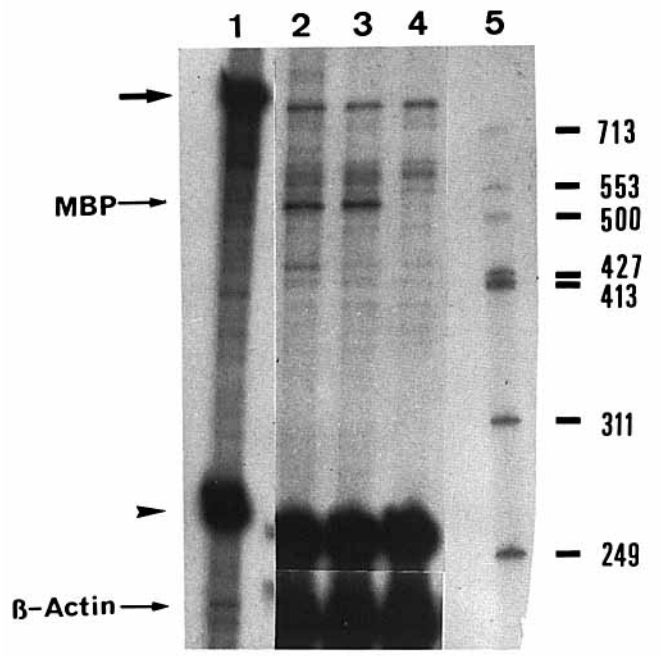

Fig. 2. Effect of T-antigen on RNA accumulation from the endogenous MBP promoter. The RNase protection assay was done with total RNA from untransfected and transfected cells. On the left, the large arrow and arrowhead indicate free probes for MBP and β-actin, respectively; MBP and β-Actin indicate the protected fragments. On the right, the numbers indicate size markers in bases. **Lane 1:** free probes for MBP and β-actin internal control for RNA recovery and transcriptional efficiency of the cells; **lane 2:** untransfected cells; **lane 3:** cells tranfected with 10 μg CMV-JCV-TΔ; **lane 4:** cells transfected with 10 μg CMV-JCV-TA; and **lane 5:** size markers.

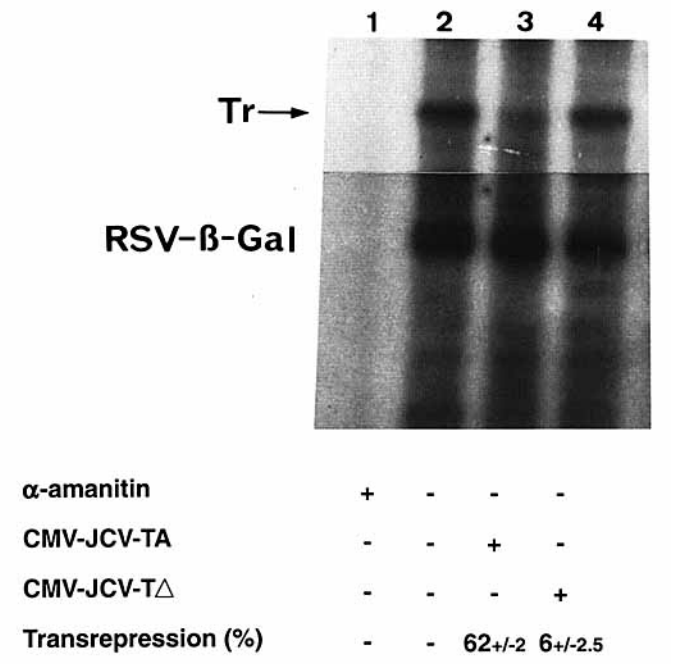

Fig. 3. Negative regulation of the MBP promoter in glioblastoma cell-free extracts. The in vitro transcription assays contained 75 μg protein from U-87 MG. The labels indicate the transcript from the MBP promoter (Tr) and the transcript from the pRSV-β-gal (RSV-β-Gal) internal cotranscription control for recovery of transcript and efficiency of the extracts. The conditions used are summarized at the bottom and the percent transrepression ± standard deviation is indicated. **Lane 1:** μg/ml α-amanitin RNA polymerase II inhibitor in untransfected cells; **lane 2:** untransfected glioblastoma cells; **lane 3:** cells transiently transfected with 10 μg CMV-JCV-TA; and **lane 4:** cells transiently transfected with 10 μg CMV-JCV-TΔ. Densitometric scanning was used to quantify the results. Other conditions, calculations, and labels were as in Figure 1B. A longer exposure was required to see the detail of the RSV-β-Gal activity.

RNase Protection Assays

Total RNA from untransfected and transfected U-87 MG was isolated using a kit and instructions with the kit (Promega, Madison, WI). Also, the RNase protection assays used a kit and instructions (Boehringer Mannheim, Indianapolis, IN). The MBP probe was derived from the 751 bp mouse MBP cDNA. β-Actin was the internal control.

In Vitro Transcription Assays

Whole cell extracts from untransfected, CMV-JCV-TA-transfected, and CMV-JCV-TΔ-transfected U-87 MG were prepared with previously described methods [Tasset et al., 1990]. *Nco*I-digested pMBPcat plasmid and *Hpa*I-digested pRSV-β-gal internal control plasmid were used as templates to assay the 576 nt CAT transcript fragment and the 386 nt β-galactosidase transcript fragment, respectively. Other methods were described previously [Kumar et al., 1993].

Immunofluorescence Assays

Indirect immunofluorescence assays were as described previously [Harlow and Lane, 1988]. U-87 MG grown on coverslips were not transfected or transfected with the CMV-JCV-TA T-antigen expressing plasmid, CMV-JCV-TΔ control plasmid, and pCMV control parental plasmid. The cells were fixed with chilled acetone:methanol (1:1) and treated with the primary monoclonal antibodies for MBP (Harlan Bioproducts, Indianapolis, IN) or JCV T-antigen [Tevethia et al., 1992]. Cells were then stained with the secondary goat anti-mouse IgG conjugated with fluorescein isothiocyanate (Jackson ImmunoResearch Lab, Inc., West Grove, PA). The staining was evaluated using a Leitz Diaplan microscope.

Western Blot Analysis

Whole cell extracts from untransfected and transfected U-87 MG glioblastoma cells were prepared, re-

Fig. 4. (Figure on following page.) Effect of T-antigen on the level of MBP produced from the endogenous gene in globlastoma cells and cell-free extracts. **A:** Protein levels of MBP and JCV T-antigen in U-87 MG. In situ immunofluorescence assays are shown. The panels represent untransfected cells and cells transiently transfected with 10 μg pCMV as a control for DNA transfection, CMV-JCV-TA, and CMV-JCV-TΔ. Staining was with the monoclonal antibodies (Abs) for MBP and T-antigen. The results were reproducible three times. **B:** Protein accumulation in cell-free extracts. The results for the four isoforms of MBP (MBP) were quantified by Western blot analysis of U-87 MG cell extracts containing 80 μg protein. The level of transfected DNA was adjusted to 20 μg with pUC19. **Lane 1:** untransfected cells; **lane 2:** cells transiently transfected with 10 μg CMV-JCV-TA; **lane 3:** cells transiently transfected with 10 μg CMV-JCV-TΔ; and **lane 4:** cells transiently transfected with 10 μg CMV parental plasmid as a negative control. Three repeated experiments were consistent.

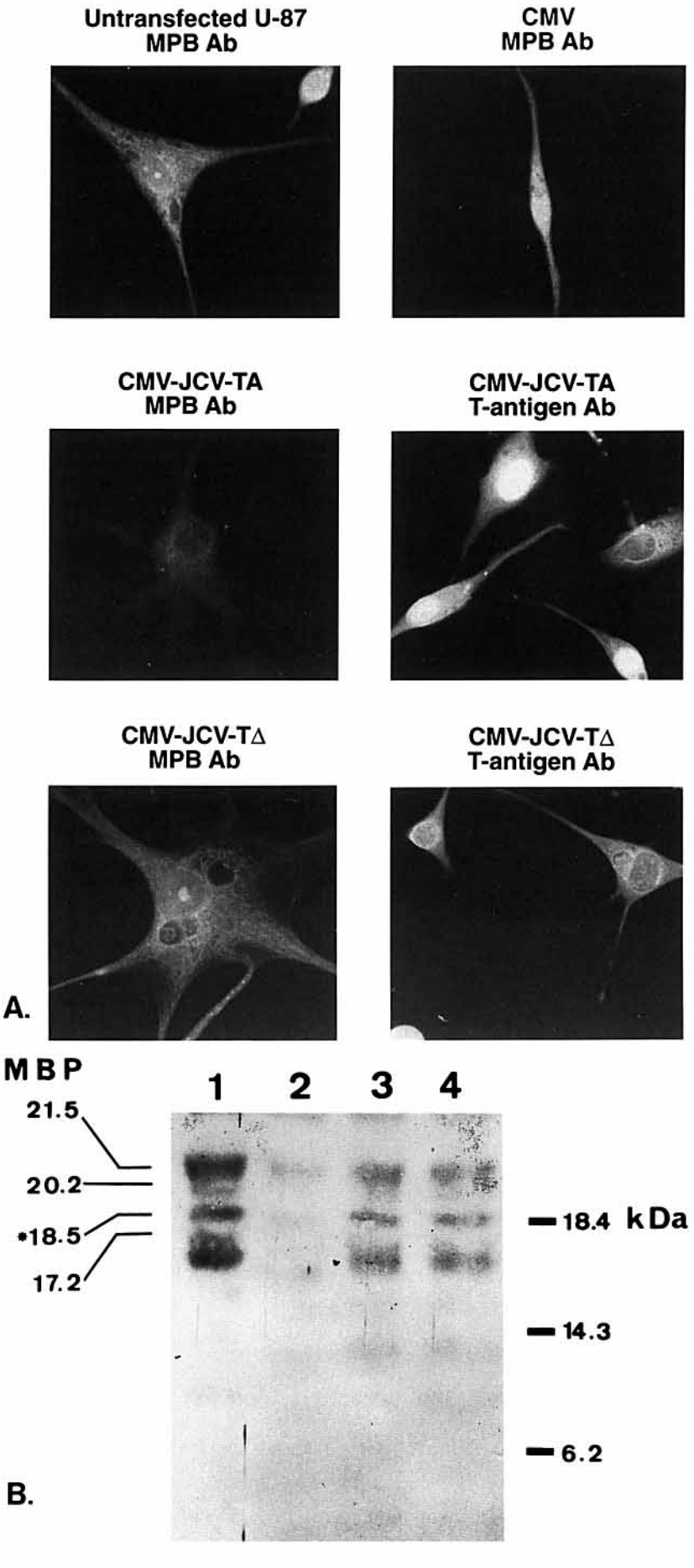

Figure 4. (Legend on preceding page.)

solved in 12% denaturing gels, and transferred to nitrocellulose membranes, as described previously [Maniatis et al., 1982]. MBP was detected on the membranes using anti-MBP monoclonal antibodies (Harlan Bioproducts), a kit, and instructions with the kit (Vector Laboratories, Burlingame, CA).

RESULTS

JCV T-Antigen Down-Regulates the Functional Transcription of the MBP Promoter in U-87 MG Human Glioblastoma Cells

The role of papovaviral T-antigen in the regulation of expression of MBP was studied previously using various systems, but the results of a typical report were confusing and contradictory [Ueno et al., 1995]. To study the effect of JCV T-antigen on the in vivo transcriptional activity of the MBP promoter, we transfected an MBP reporter plasmid into U-87 MG human glioblastoma cells with or without the CMV-JCV-TA T-antigen expressing plasmid. The pMBPcat reporter plasmid in the sense orientation showed efficient activity in U-87 MG (Fig. 1B). The MBP promoter cloned in the antisense orientation showed no detectable activity (data not shown). Cotransfection of the sense orientation plasmid with CMV-JCV-TA led to a 2.5-fold reduction of the activity of the MBP promoter (Fig. 1B,C). Further, the presence of T-antigen in these transfected cells was determined by Western blot analysis for the U-87 MG cotransfected with CMV-JCV-TA; also, cotransfection with JCV-pML containing the complete JCV genome [Nakshatri et al., 1990] led to a significant down-regulation of the MBP promoter (data not shown). In contrast, no effect on activity due to cotransfection was observed with CMV-JCV-TΔ (Fig. 1B,C) containing a deletion of most of the T-antigen coding sequences [Nakshatri et al., 1990]. As a control for the integrity of the transcriptional machinery of U-87 MG and the transfection efficiency, the in vivo activity of the promoter of the pRSVcat Rous sarcoma virus (RSV) plasmid was examined. The expression from the RSV promoter was not reduced by T-antigen (Fig. 1B,C). These results indicated that T-antigen transrepressed the activity of the MBP promoter in glioblastoma cells.

Effect of JCV T-Antigen on Accumulation of MBP mRNA From the MBP Promoter in Human Glioblastoma Cells

RNase protection assays were used to distinguish whether the effect of T-antigen was due to transcriptional control of the endogenous promoter or any possible translational effects, such as the function of the translation control elements in the 5′ untranslated sequences of the MBP mRNA in the MBP promoter vector [Verdi and Campagnoni, 1990]. Therefore, the production of MBP RNA from the endogenous gene was assayed, using total RNA from the untransfected U-87 MG or cells transfected with the expression of JCV T-antigen. The effect was similar to that for the functional expression of the MBP promoter. Total RNA from untransfected cells yielded a significant level of the expected 751 bp protected fragment (Fig. 2, lane 2). Transfection of CMV-JCV-TA greatly reduced the level of MBP RNA (Fig. 2, lane 4). To confirm that the effect was due to the expression of intact T-antigen and that the U-87 MG transcriptional machinery was not compromised, the CMV-JCV-TΔ expression control was used. Convincingly, there was no effect for this control (Fig. 2, lane 3) relative to the untransfected U-87 MG. Additionally, the RNA for transfections with the CMV vector was used as a similar control. The result showed that the CMV vector also had no effect on the MBP RNA level (data not shown).

Effect of JCV T-Antigen on Transcription in Glioblastoma Cell-Free Extracts

Although the RNase protection assays showed a marked effect of T-antigen on the MBP RNA level, the results may have been complicated by the presence of preexisting mRNA in the cells or by the more rapid turnover of MBP mRNA due to T-antigen. Therefore, the effect of T-antigen on the rate of RNA production from the MBP promoter was also quantified by run off in vitro transcription assays (Fig. 3). As expected, T-antigen nonexpressing U-87 MG produced substantial levels of MBP mRNA (Fig. 3, lane 2). In the presence of extract from U-87 MG glioblastoma cells expressing T-antigen, transcription of the pMBPcat MBP reporter plasmid was reduced 2.5-fold (Fig. 3, lane 3). Similar to the in vivo results for the functional expression of MBP from the MBP reporter vector (Fig. 1B,C) and for the accumulation of MBP transcripts from the endogenous MBP gene (Fig. 2, lane 3), transcription in the cell-free system was not affected when CMV-JCA-TΔ was used (Fig. 3, lane 4). Whereas the transcriptional competency of the extracts might have been negatively effected by T-antigen, the three results for the pRSV-β-gal control (Fig. 1), the β-actin control (Fig. 2), and the pRSV-β-gal control (Fig. 3) consistently indicated that such effects were not a factor.

JCV T-Antigen Similarly Reduces the Level of MBP Produced From the Endogenous Gene in Glioblastoma Cells

To investigate the effect of regulation by T-antigen on the cellular location and level of MBP produced from the endogenous MBP gene resident in the U-87 MG, the cells were transfected with the CMV-JCV-TA T-antigen expressing plasmid. MBP was readily detected by immunofluorescence assays of the untransfected cells (Fig. 4A). The cellular staining pattern was consistent with the expected, granular cytoplasmic staining of MBP. The MBP staining pattern was visibly abnormal and the level was reduced in U-87 MG producing T-antigen from CMV-JCV-TA and was reminiscent of the results in the comparable cell type in the CNS [Allinquant et al., 1991]. As for the three assays described above, the CMV-JCV-TΔ expression vector containing the T-antigen delection had no effect on MBP. Transfection with the parental pCMV control plasmid also had no effect on the level of MBP that was produced from the resident MBP gene in U-87 MG. To examine the expression of T-antigen, cells were also stained using T-antigen antibody. Many cells gave the expected nuclear staining pattern of T-antigen expressed from CMV-JCV-TA (Fig. 4A). As a control,

cells were stained doubly for T-antigen and a glial-specific marker and the results showed that glial cells represented 90% of the transfected cells expressing T-antigen (data not shown). The CMV-JCV-TΔ control plasmid was negative for the expression T-antigen (Fig. 4A).

To reexamine and to quantify the effect of CMV-JCV-TA on the production of MBP from the endogenous U-87 MG MBP gene, Western blots of the glioblastoma cell extracts were used (Fig. 4B). The extracts of untransfected cells contained a substantial amount of MBP (Fig. 4B, lane 1). Furthermore, transfection of CMV-JCV-TA, which was shown to result in the production of the viral protein (Fig. 4A), also led to a substantial reduction in the level of endogenous MBP (Fig. 4B, lane 2). These assays confirmed quantitatively the results of Figure 4A, panel 2. However, a moderate reduction of MBP in U-87 MG was seen by the transfection of CMV-CV-TΔ (Fig. 4B, lane 3) and by the transfection of the CMV plasmid control for the effect on the transcriptional machinery (Fig. 4B, lane 4). Therefore, the CMV promoter may down-regulate translation, since RNase protection showed no such effect (Fig. 2). However, a marked reduction was seen if the plasmid also produced T-antigen (Fig. 4B), which is consistent with the results of the other four assays. Taken together, the results showed the negative effect of T-antigen on the level of functional transcription of the MBP promoter, the level and the rate of production of MBP mRNA, and the level of MBP produced from the endogenous MBP gene.

DISCUSSION

The mechanism by which the neurotropic human JCV induces demyelination in the PML lesions of the CNS has been elusive. Studies showed that transgenic mice harboring the JCV early region coding for T-antigen developed severe dysmyelination in the CNS cells, but not in the PNS cells, containing high levels of T-antigen [Small et al., 1986]. In addition, a down-regulation of the important myelin protein MBP was observed in these transgenic mice [Small et al., 1986; Haas et al., 1994]. This effect was attributed to the inhibition of the maturation of oligodendrocytes by T-antigen [Trapp et al., 1988]. Our results showed that the cellular distribution pattern of MBP produced without and with the effect of T-antigen expression in U-87 MG glioblastoma cells (Fig. 4A) was similar to the pattern in the oligodendrocytes of the CNS in normal and shiverer mice, respectively [Allinquant et al., 1991]. Thus, these results indicate the fidelity of the glioblastoma cell model system. Data with this model present evidence that JCV T-antigen represses the MBP promoter, leading to lower levels of MBP. The possibility of an additional regulation of translation of MBP cannot be completely excluded. However, the similar levels of the in vivo MBP promoter functional activity, of the MBP mRNA, of the rate of mRNA production and of MBP, indicated that the regulation by JCV T-antigen occurred at the transcriptional level in glioblastoma cells. Although the related simian virus 40 (SV40) polyomavirus is not neurotropic or associated with PML in the monkey natural host or in humans, a recent report used in vivo expression and in vitro translation assays of MBP to show that translation was not inhibited by SV40 T-antigen [Ueno et al., 1995].

The polyomavirus T-antigens are complex multifunctional proteins that repress transcription of cellular genes by several pathways. Based on this information, JCV T-antigen might negatively regulate MBP gene expression indirectly, cooperatively with other factors or directly. First, JCV T-antigen interacts with the p53 and pRb cellular tumor suppressor gene proteins, leading to the loss of the pleiotropic functions of these transcription-regulating proteins [Haggerty et al., 1989; Dyson et al., 1990]. The SV40 T-antigen also interacted physically and functionally with the c-Jun transcription factor, resulting in the indirect down-regulation of the MBP promoter by T-antigen [Bharucha et al., 1994]. Second, JCV T-antigen may interact with and alter the functional binding of cellular proteins(s) to repress the MBP promoter, as was shown for the inhibition by SV40 T-antigen of the AP-2 transcriptional activator [Mitchell et al, 1987]. Further , JCV T-antigen was shown to transregulate JCV_L by synergistic interaction with the tat protein of the AIDS virus [Chowdhury et al., 1990]. Third, JCV T-antigen may down-regulate the MBP promoter directly. For the partially homologous SV40 T-antigen, binding to the GAGGC sequence present in the SV40 promoter and in the cellular genome was demonstrated. Thereby, viral and cellular gene expression was altered [Tjian, 1978; Lane et al., 1985; Gruss et al., 1988]. In addition, the oligodendroglial cells in the CNS of transgenic mice containing and expressing SV40 T-antigen displayed hypomyelination [Jensen et al., 1993]. JCV contains GAGGC in the viral regulatory region [Chang et al., 1994], suggesting a similar function for JCV T-antigen. Interestingly, clusters of this sequence are present in the MBP promoter [Miura et al., 1989]. Therefore, JCV T-antigen may similarly bind directly to the GAGGC in the MBP promoter, leading to reduced levels of myelin due to the down-regulation of the MBP promoter in PML patients.

PML is the major pathology of JCV polyomavirus. Another feature of JCV is that the oncogenicity of this tumor virus is neurotropic. The neuro-oncogenic property of JCV may be explained by the production of T-antigen and the subsequent inactivation, by the T-antigen that is expressed in CNS cells, of p53 and pRb [Haggerty et al., 1989; Dyson et al., 1990]. Also, monkeys were shown to form brain tumors when infected with JCV [London et al., 1978]. Furthermore, an immune-competent PML patient succumbed to PML lesions with infected oligodendrocytes and astrocytes from which multiple malignant astrocytomas originated [Sima et al., 1983]. Similarly, hyperplastic and neoplastic-like astrocytes were reported for PML earlier (Åström et al., 1958.

PML has increased dramatically in an epidemic association with AIDS [Quinlivan et al., 1992]. In addition, the role of JCV in the pathology of PML was clearly documented in the absence of AIDS [Major et al., 1992]. Polyomaviral inclusion bodies and lytic infection of oligodendroglial cells were detected in all cases of PML, in which there is a sharp reduction of the myelin sheath [Padgett et al., 1977; for reviews see Johnson, 1983; Firsque and White, 1992; Major et al., 1992]. T-antigen

may initiate PML by acting as a transregulator to down-regulate MBP transcription (this report) indirectly, co-operatively, or directly, as described above. The down-regulation of MBP transcription in human glioblastoma cells by JCV T-antigen suggests a model system to study PML. Importantly, our system demonstrated quantitatively similar down-regulation of the protein produced from the endogenous MBP gene, implicating a negative transcriptional regulatory mechanism for the demyelination observed in the CNS of PML patients.

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REFERENCES

Allinquant B, Staugaitis SM, D'Urso D, Colman DR (1991): The ectopic expression of myelin basic protein isoforms in *shiverer* oligodendrocytes: Implications for myelinogenesis. Journal of Cell Biology 113:393–403.

Åström K-E, Mancall EL, Richardson EP (1958): Progressive multifocal leukoencephalopathy: Hitherto unrecognized complication of chronic lymphatic leukaemia and Hodgkin's disease. Brain 81:93–111.

Bharucha VA, Peden KWC, Tennekoon GI (1994): SV40 large T-antigen with c-Jun downregulates myelin P_0 gene expression: A mechanism for papovaviral T-antigen-mediated demyelination. Neuron 12: 627–637.

Brophy PJ, Boccaccio GL, Colman DR (1993): The distribution of myelin basic protein mRNAs within myelinating oligodendrocytes. Trends in Neurosciences 16:515–521.

Chang CF, Tada H, Khalili K (1994): The role of a pentanucleotide repeat sequence, AGGGAAGGGA, in the regulation of JC virus DNA replication. Gene 148:309–314.

Chowdhury M, Taylor JP, Tada H, Rappaport J, Wong-Staal F, Amini S, Khalili K (1990): Regulation of the human neurotropic virus promoter by JCV-T-antigen and HIV-1 tat protein. Oncogene 5:1737–1742.

Demel RA, London Y, Geurts Van Kessel WSM, Vossenberg FGA, Van Deenen LLM (1973): The specific interaction of myelin basic protein with lipids at the air-water interface. Biochimica et Biophysica Acta 311:507–519.

Dyson N, Bernards R, Friend SH, Gooding LR, Hassel JA, Major EO, Pipas JM, Vandyke T, Harlow E (1990): Large T-antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. Journal of Virology 64:1353–1356.

Frisque RJ, White FA (1992): The molecular biology of JC virus, causative agent of progressive multifocal leukoencephalopathy. In Roos RP (ed): "Molecular Neurovirology." Totowa, NJ: Humana Press.

Gorman CM, Moffat LF, Howard BH (1982): Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Molecular and Cellular Biology 2:1044–1051.

Gruss C, Wetzel E, Baack M, Mock U, Knippers R (1988): High affinity SV40 T-antigen binding sites in the human genome. Virology 167:349–360.

Haas S, Haque NS, Beggs AH, Khalili K, Knobler RL, Small JA (1994): Expression of the myelin basic protein gene in transgenic mice expressing human neurotropic virus, JCV, early protein. Virology 202:89–96.

Haggerty S, Walker D, Frisque RJ (1989): JC virus-simian virus 40 genomes containing heterologous regulatory signals and chimeric early regions: Identification of regions restricting transformation by JC virus. Journal of Virology 63:2180–2190.

Harlow E, Lane D (1988): Cell staining. In: "Antibodies: A Laboratory Manual." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 359–420.

Jensen NA, Smith GM, Shine HD, Garvey JS, Hood L (1993): Distinct hypomyelinated phenotypes in MBP-SV40 large T transgenic mice. Journal of Neuroscience Research 34:257–264.

Johnson RT (1983): Evidence for polyomavirus in human neurological diseases. In Sever JL, Madden DL (eds): "Polyomaviruses and Human Neurological Diseases." New York: Alan R. Liss, pp 183–190.

Kumar KU, Pater A, Pater MM (1993): Human JC virus perfect palindromic nuclear factor-1 binding sequences important for glial cell-specific expression in differentiating embryonal carcinoma cells. Journal of Virology 67:572–576.

Lane DP, Simanis V, Bartsch R, Yewdell J, Gannan J, Mole S (1985): Cellular targets for SV40 large T-antigen. Proceedings of the Royal Society of London (B) 266:25–42.

London WT, Houff SA, Madden DL, Fuccillo DA, Gravell M, Wallen WC, Palmer AE, Sever JL, Padgett BL, Walker DL, ZuRhein GM, Ohashi T (1978): Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). Science 201:1246–1249.

Major EO, Amemiya K, Tornatore C, Houff S, Berger J (1992): Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clinical Microbiological Reviews 5:49–73.

Maniatis T, Fritsch EF, Sambrook J (1982): "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Mitchell PJ, Wang C, Tjian R (1987): Positive and negative regulation of transcription in vitro: Enhancer-binding protein AP-2 is inhibited by SV40 T-antigen. Cell 50:847–861.

Miura M, Tamura T-A, Aoyama A, Mikoshiba K (1989): The promoter elements of the mouse myelin basic protein gene function efficiently in NG108-15 neuronal/glial cells. Gene 75:31–38.

Nakshatri H, Pater A, Pater MM (1990): Activity and enhancer binding factors for JC virus regulatory elements in differentiating embryonal carcinoma cells. Virology 177:784–789.

Padgett BL, Rogers CM, Walker DL (1977): JC virus, a human polyomavirus associated with progressive multifocal leukoencephalopathy: Additional biological characteristics and antigenic relationships. Infection and Immunity 15:656–662.

Quinlivan EB, Norris M, Bouldin TW, Suzuki K, Meeker R, Smith MS, Hall C, Kenney S (1992): Subclinical central nervous system infection with JC virus in patients with AIDS. Journal of Infectious Diseases 166:80–85.

Roach A, Boylan K, Horvath S, Prusiner SB, Hood LE (1983): Characterization of cloned cDNA representing rat myelin basic protein: Absence of expression in brain of shiverer mutant mice. Cell 34:799–806.

Sima AAF, Finkelstein SD, McLachlan DR (1983): Multiple malignant astrocytomas in a patient with spontaneous progressive multifocal leukoencephalopathy. Annals of Neurology 14:183–188.

Small JA, Scangos GA, Cork L, Jay G, Khoury G (1986): The early region of human papovavirus JC induces dysmyelination in transgenic mice. Cell 46:13–18.

Tasset D, Tora L, Fromental C, Scheer E, Chambon P (1990): Distinct classes of transcriptase activating domains function by different mechanisms. Cell 62:1177–1187.

Tevethia SS, Epler M, Georgoff I, Teresky A, Marlow M, Levine AJ (1992): Antibody response to human papovavirus JC (JCV) and simian virus 40 (SV40) T antigens in SV40 T antigen-transgenic mice. Virology 190:459–464.

Tjian R (1978): The binding site of SV40 DNA for a T-antigen related protein. Cell 13:165–179.

Trapp BD, Small JA, Pulley M, Khoury G, Scangos GA (1988): Dysmyelination in transgenic mice containing JC virus early region. Annals of Neurology 23:38–48.

Ueno S, Foster L, Hifumi GT, Tennekoon GI, Campagnoni AT (1995): The simian virus 40 large T-antigen does not inhibit translation of the 14-kDa myelin basic protein mRNA in reticulocyte lysates or in transfected cells. Journal of Neurochemistry 64:928–931.

Verdi JM, Campagnoni AT (1990): Translational regulation by steroids. Identification of a steroid modulatory element in the 5′-untranslated region of the myelin basic protein messenger RNA. Journal of Biological Chemistry 265:20314–20320.